FACULTY OF BIOSCIENCE ENGINEERING

# DESIGNER CELLULOSOMICS FOR A CUSTOMIZED CONVERSION OF LIGNOCELLULOSIC BIOMASS

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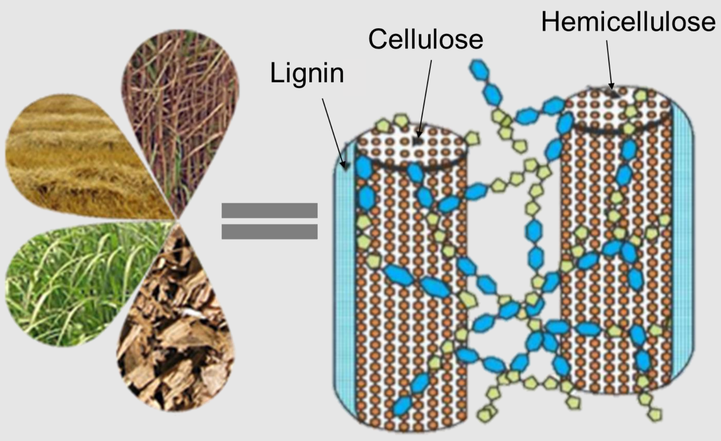
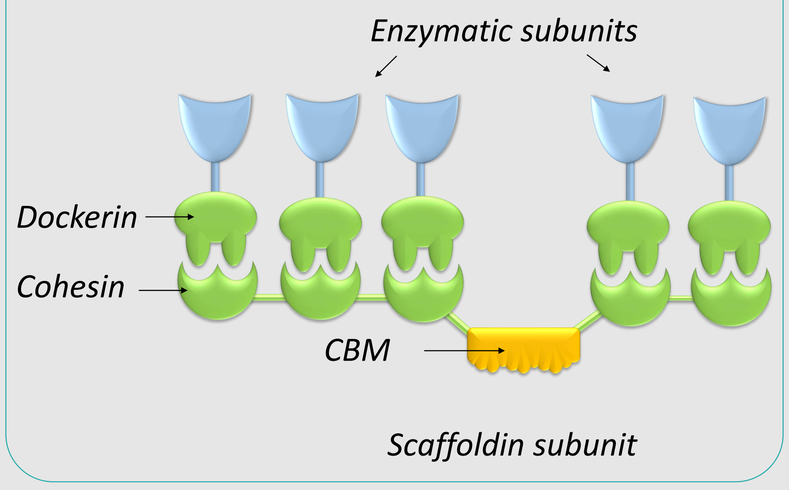
## Background

**Lignocellulose** is a major component of plant cell walls and is present in a large amount of waste and side streams. Efficient use of this component as a resource is one of the most important challenges in the field of industrial biotechnology.




**Cellulosomes** are multi-enzyme complexes produced by specific micro-organisms that feed on plant cell wall carbohydrates. All catalytic modules with complementary functions are close to each other, enhancing their synergism.

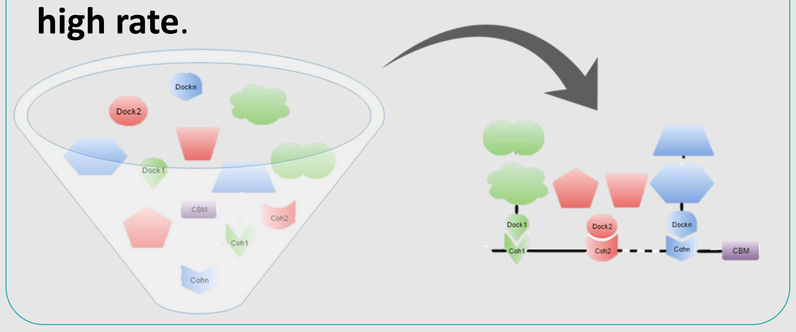



## Aim

Use knowledge about the naturally occurring enzyme complexes to create engineered **designer cellulosomes (DC)** with the potential to degrade all lignocellulosic components.

**Current drawback**: use of **standard cloning techniques** makes the construction process tedious and allows the creation and analysis of only one or a few designer cellulosome(s) at a time.

Our lab has developed a **DNA assembly method**, **VersaTile Shuffling (VTS)**, which enables us to create **modular proteins** at a **high rate**.

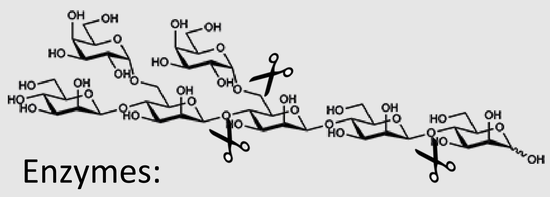



## Results

### Step I
Choice of substrate

**Galactomannan**

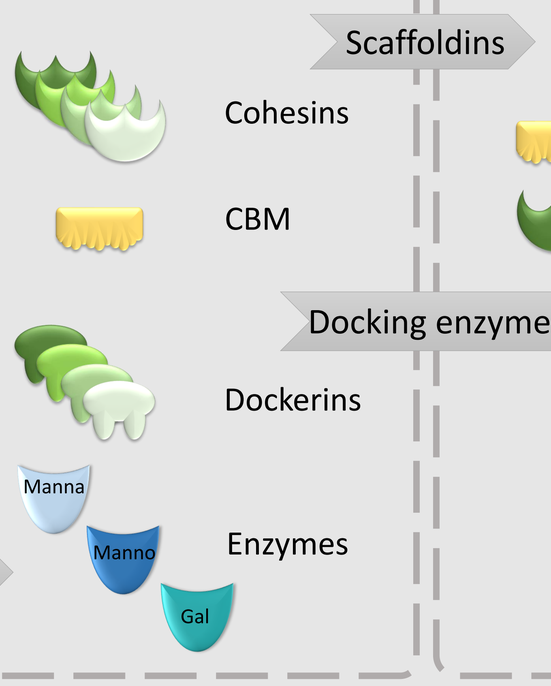
- No galactomannan degrading cellulosome described in literature yet
- Predominant hemicellulose of hardwoods
- Waste in food supply chain



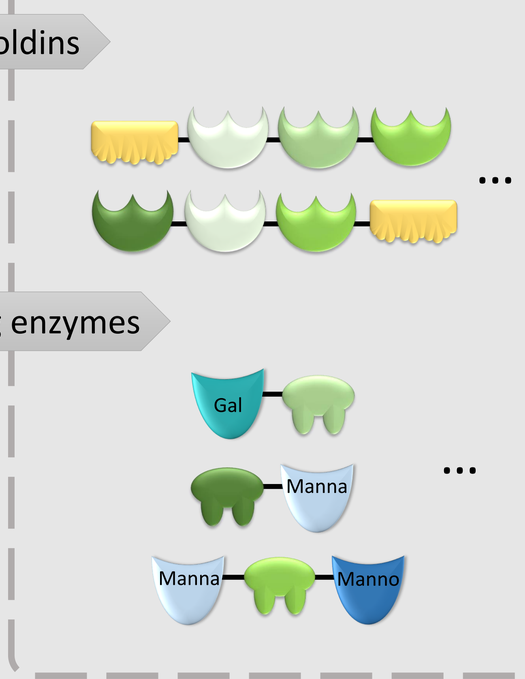
Enzymes:

- β-1,4-mannanase
- β-1,4-mannosidase
- α-1,6-galactosidase

### Step II
Creating building blocks

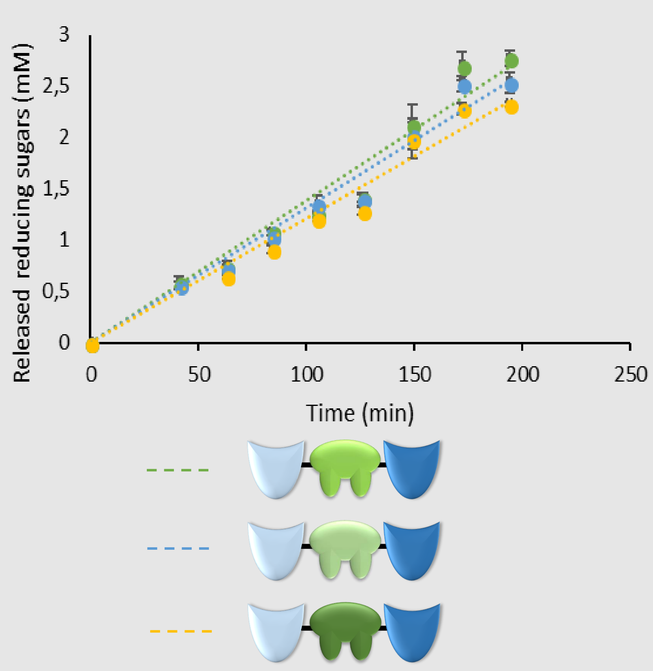



### Step III
VTS assembly

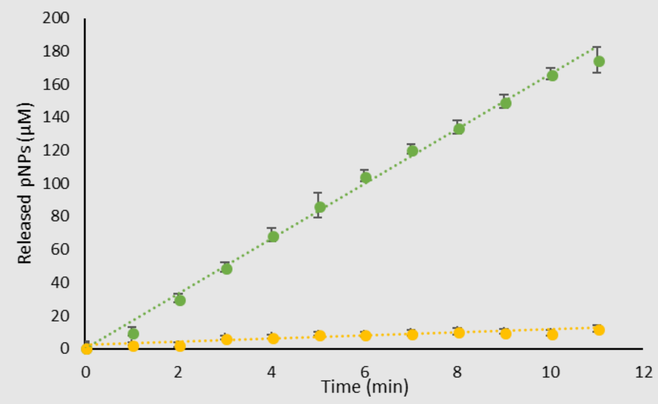



### Step IV
Systematic parameter analysis

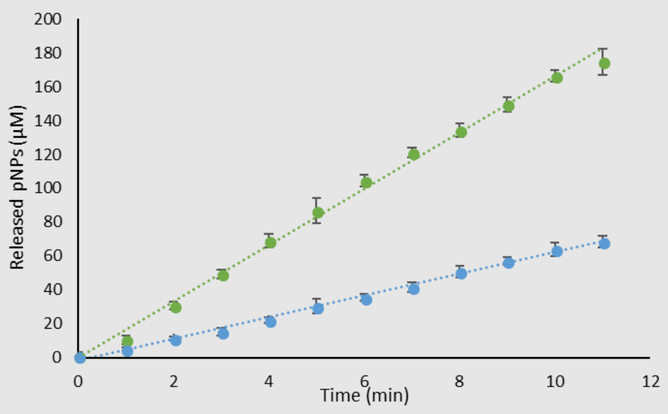
**Parameter 1**: Dockerin type



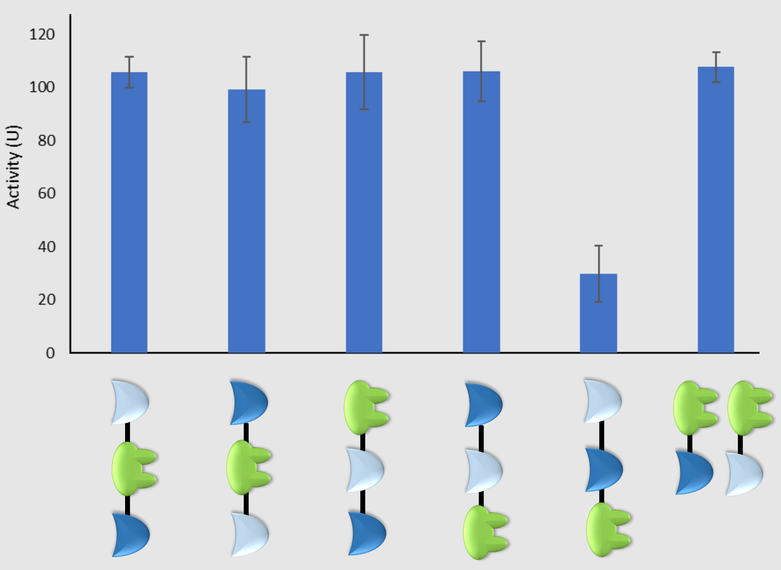

**Parameter 2**: Dockerin position



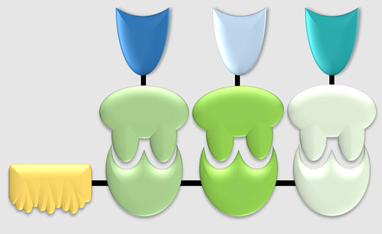

**Parameter 3**: Influence natural CBM




**Parameter 4**: bicatalytic vs monocatalytic




### Step V
Determination of optimal docking enzyme and scaffoldin combination



- High number of variables influences DC performance
- Preliminary assays are essential
- Quick and easy assembly method such as VTS allows efficient evaluation of all variables

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